Effects of vitamin B-6 deficiency and aging on pyridoxal 5′-phosphate levels and glycogen phosphorylase activity in rats

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Muscle, liver, brain, and plasma pyridoxal 5′-phosphate (PLP) concentrations and muscle, liver, and brain glycogen phosphorylase (GPase) activity were determined during vitamin B-6 deficiency and aging. Fischer 344, male rats, aged 15 weeks, and 6, 15, and 25.5 months at the end of the 12-week study, were divided into diet groups of ad libitum controls, deficient or pair-fed controls. Plasma PLP levels in 25.5-month-old control rats were lower ($P \leq 0.05$) than the other three age groups which were slightly, but not significantly different from each other. Plasma PLP of deficient rats was dramatically decreased ($P \leq 0.05$) in all age groups. Muscle PLP was decreased ($P \leq 0.05$) in all vitamin B-6 deficient rats. Liver and brain PLP concentrations were decreased ($P \leq 0.05$) in 15 week, 6 month, and 15 month deficient rats only. Tissue PLP levels were correlated ($P \leq 0.01$) with plasma PLP. Vitamin B-6 deficiency resulted in decreased glycogen phosphorylase activity ($P \leq 0.05$) in muscle, liver, and brain of 15 week rats, and in muscle and liver of 6 month rats. Reduction in food intake did not account for the reported changes during vitamin B-6 deficiency. A significant correlation between tissue glycogen phosphorylase activity and tissue PLP levels was observed. Vitamin B-6 deficiency significantly lowered tissue PLP and phosphorylase activity, but the decrease in each was less marked with increasing age.

Keywords: vitamin B-6; aging; pyridoxal 5′-phosphate; glycogen phosphorylase; muscle; liver; brain

Introduction

The enzyme glycogen phosphorylase (GPase) requires pyridoxal 5′-phosphate (PLP) to maintain its role as the key control enzyme in the process of glycogen degradation to glucose-1-phosphate.[1] The requirement for glycogen as an energy source varies greatly between tissues. Normal glycogen levels in mouse liver, muscle, and brain are in the proportions of approximately 100:10:1, and are strictly regulated to maintain metabolic homeostasis.[2]

The importance of PLP in maintaining the activity of GPase has been demonstrated in liver,[3] skeletal muscle,[4] and brain.[5] In all of these studies, vitamin B-6 was excluded from the diets of young, growing animals, in order to deplete the animals' stores of PLP. These studies showed that vitamin B-6 deficiency decreased GPase activity in young growing animals; however, it is not known whether a similar effect would be observed in adult or aged animals.

There is evidence in the literature that young and aged animals exhibit differences in the metabolism of vitamin B-6 when fed similar diets.[6–9] One possible mechanism for this effect in animals is that age may decrease the availability of the phosphorylated cofactor forms of vitamin B-6. The situation is more complicated in humans since diets vary greatly between individuals. The intake of vitamin B-6 in elderly individuals was shown to be below the current Recommended Dietary Allowances (RDA).[10,11] If the elderly do have lower vitamin B-6 status, then functional deficits in PLP-dependent enzymes such as glycogen phosphorylase may exist. The present study was undertaken to examine the effect of vitamin B-6 deficiency on PLP concentration and PLP-dependent gly-

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cogen phosphorylase activity in skeletal muscle, liver, and brain in rats of different ages.

Materials and methods

Animals and diets

Fischer rats aged 3 months (n = 30), 12 months (n = 30), and 18 months (n = 30) were obtained from the National Institute of Aging (NIA) colony (Harlan Sprague Dawley, Inc., Indianapolis, IN). Fischer 344, male, 3-week-old rats (n = 30) were purchased from Charles River Laboratories (Kingston, NJ), since young rats were not available from the NIA colony. Rats were housed individually in stainless steel cages in a temperature (22–24°C) and light-controlled (12 h/d) room. Water was available ad libitum. All rats were allowed to acclimate for at least five days and were fed Prolab 3000 (Agway, Inc.) during this time. The 18-month rats were kept at these conditions until they began the study at 23 months of age due to the limited availability and expense of obtaining aged rats. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Tufts University.

Animals were grouped by weight and then assigned randomly to dietary groups. The three dietary groups were: (1) ad libitum vitamin B6-sufficient controls (ALC) (7 mg pyridoxine hydrochloride (PN:HCl) were added per kg of diet), (2) ad libitum vitamin B6-deficient (DEF) (0 mg PN:HCl were added per kg of diet), and (3) pair-fed controls (PF). The 3-week, 3-month, and 12-month rats were fed for 12 weeks, but the study on 23-month rats ended at 10 weeks due to high morbidity, unrelated to diet. The data were reported using the age of the rat at the conclusion of the study: 15 weeks, 6 months, 15 months, and 25.5 months, respectively. Components of the experimental ad libitum control diet were purchased individually (Teklab; Madison, WI), then mixed. The diet consisted of the following (g/100 g of diet): vitamin-free casein, 25.0; DL-methionine, 0.30; cornstarch, 15.0; sucrose, 45.0; Cellufil (U.S. Biochemical, Cleveland, OH) 5.0; corn oil, 5.0; American Institute of Nutrition (AIN)[12] mineral mix, 3.5; AIN-76A[13] vitamin mix, 1.0; and choline bitartrate, 0.20. The AIN-76 mineral mix was purchased from a commercial vendor (Teklab, Madison, WI). The total vitamin B-6 concentration in the semi-purified vitamin B-6 sufficient (5.91 mg of vitamin B-6/kg diet) and deficient (0.07 mg of vitamin B-6/kg diet) experimental diets was confirmed by analysis using the method of the Association of Official Analytical Chemists.[14]

Preparation of samples

Blood was collected at baseline, which was one day prior to starting the experimental diet, and again at the end of the experimental period. Rats were fasted for 14 hours prior to having their blood drawn from the retro-orbital sinus using anesthesia. Blood was collected, processed, and stored frozen at −70° C until analysis.[9] Rats were fasted for 14 hours and sacrificed by decapitation one week after the final blood collection, in order to avoid possible effects of anesthesia on glycogen phosphorylase activity. Brain, liver, and approximately 100 mg of the gastrocnemius muscle were removed rapidly and frozen in liquid nitrogen. Organs were lyophilized at −40° C, ground to a powder, weighed, and stored frozen until analysis. Tissues were lyophilized so that homogeneous samples of each tissue could be obtained for both PLP and GPase analysis. Body weights were monitored weekly and food intake was measured every two days for all but the 6-month ALC rats. The 6-month study was carried out slightly earlier than the other age groups and at this time we did not realize that food intake of control rats would be of interest to the study. Several rats of all ages developed infections from the blood draw procedure and were euthanized. Many of the older rats were sacrificed when tumors or abcesses were found.

Pyridoxal-5-phosphate analysis

Brain homogenates were prepared by combining 50 mg of lyophilized brain tissue with 2.8 mL of deionized water and 0.2 mL of 10% m-phosphoric acid. Approximately 10 mg of liver or muscle were homogenized with 1.0 mL of 0.08 M sodium phosphate buffer (pH 7.4) and deproteinized with 10% trichloroacetic acid. Homogenates were sonicated for 30 seconds (Branson Sonicator Model 350 with microtip, Danbury, CT). Pyridoxal-5′-phosphate (PLP) concentrations in plasma, brain, liver, and muscle were determined by a modified procedure of the L-tyrosine apodecarboxylase method of Chabner and Livingston.[15] Details of assay modifications have been described previously.[16,17] Tissue PLP concentrations are expressed as nmol/mg protein. Materials for this assay included L-[1-^{14}C]-Tyrosine (specific activity 53.4 mCi/mmol), Econofluor (New England Nuclear, Boston, MA), L-tyrosine decarboxylase apoenzyme and pyridoxal 5′-phosphate (Sigma Chemical Co., St. Louis, MO).

Glycogen phosphorylase activity

Lyophilized tissue (25 mg) was homogenized with 1.0 mL of 50 mM alpha-(N-Morpholine) ethanesulfonic acid (pH 6.1). Total glycogen phosphorylase activity, the amount of activity present when assaying in the presence of AMP (5mmol/L), was analyzed using the method of Gilboe et al.[18] Glycogen phosphorylase activity was expressed as nkat/mg protein. Materials for this assay included glucose-1-phosphate (G-1-P), 5′-adenosine monophosphate (AMP) and phosphorylase a (purified), which were purchased from Sigma Chemical Co. (St. Louis, MO). Rabbit liver glycogen (Sigma Chemical, Co., St. Louis, MO) was purified using a column of mixed-bed ion-exchange resin, Amberlite MB-3 (Sigma Chemical Co., St. Louis, MO).

Protein analyses

The protein concentration of each tissue homogenate was determined by the method of Bradford[19] using bo-

vine serum albumin as a standard. Coumassie Brilliant Blue G-250 protein assay was purchased from Bio-Rad (Richmond, CA).

Statistical analyses

Statistical analyses were done using SAS software (SAS Institute Inc., Cary, NC). A two-way analysis of variance (ANOVA) that adjusted for unbalanced designs (General Linear Models) was used to determine significant effects of diet and age. If there was a significant effect of age ($P \leq 0.05$), the age group means were examined further with Tukey's HSD (honestly significant difference) test.[20] A significant effect of diet ($P \leq 0.05$) within an age group was examined first by an unpaired two-tailed comparison t test between ALC and PF rats. If there was no significant difference ($P > 0.05$) between these control groups, then pair-fed and deficient rats were compared by a two-tailed paired comparisons t test, and the pair-fed rats were then used as the control group in the discussion of results.

Results

Body weights and food intake

Body weight and food intake were monitored during vitamin B-6 deficiency, in order to verify the deficient state. Body weights of only the 15-week and 6-month-old rats were lowered in vitamin B-6 deficiency or pair-feeding (*Table 1*). The PF and DEF rats of both ages had significantly lower ($P \leq 0.05$) body weights than ALC rats. Growth was depressed by decreased food intake in 15-week DEF rats shown by their significantly lower ($P \leq 0.05$) body weights than PF rats.

The food intakes of 15-week DEF rats were significantly lower ($P \leq 0.05$) than intakes of ALC rats of the same age (*Table 1*). There were no differences in average daily food consumption by the final week for 15- and 25.5-month old rats. No food intake was recorded for 6-month ALC rats (see Materials and methods). However, food intake of 6-month DEF rats was significantly lower ($P \leq 0.05$) at the end of the study (9.3 ± 0.1) when compared to intake during the first week (12.3 ± 1.2).

If food intake is expressed as grams of food consumed per 100 grams of body weight, the 15-week DEF rats consumed more food (6.1g) than either the ALC (4.9g) or PF (4.7g) rats. This indicates that the food consumed by the young, growing DEF rats was converted less efficiently to body mass than for the ALC or PF rats. The "feed efficiency" was not affected by diet in any of the other age groups.

Plasma PLP

Plasma PLP values for all dietary treatments are reported in *Table 2*. There was a significant effect ($P \leq 0.05$) of pair-feeding on plasma PLP only in the 15-week rats, which was 76% of the ALC value. However, plasma PLP levels of 25.5 month ALC and PF rats were significantly lower ($P \leq 0.05$) and only 45% of the PLP levels of other ages. Plasma PLP was decreased significantly ($P \leq 0.05$) in DEF rats compared to PF rats of all ages studied. The 15-month DEF rats had significantly higher ($P \leq 0.05$) plasma PLP than other age groups.

Table 1 Body weight and food intake of rats

Group	Body Weight Initial (g)	Body Weight Final (g)	Mean Food Intake of Final Week[2] (g/day)
15 Weeks			
ALC	62 ± 3 (10)	232 ± 8 (7)	11.3 ± 0.6
DEF	62 ± 3 (10)	129 ± 4 (8)**	7.9 ± 0.3***
PF	63 ± 3 (10)	169 ± 7 (8)**	7.9 ± 0.3
6 Months			
ALC	281 ± 8 (10)	302 ± 10 (7)	NA
DEF	279 ± 10 (10)	265 ± 10 (9)**	9.3 ± 0.1
PF	258 ± 12 (10)	277 ± 7 (9)*	9.3 ± 0.1
15 Months			
ALC	404 ± 10 (10)	385 ± 10 (7)	13.7 ± 2.6
DEF	410 ± 9 (10)	350 ± 9 (7)	13.7 ± 0.2
PF	409 ± 11 (10)	386 ± 8 (7)	13.7 ± 0.2
25.5 Months			
ALC	387 ± 12 (10)	380 ± 13 (5)	13.6 ± 1.3
DEF	417 ± 14 (10)	345 ± 14 (5)	12.3 ± 1.5
PF	399 ± 15 (10)	349 ± 11 (5)	12.3 ± 1.5

Note. All data are given as mean ± SEM. The final mean daily food intake values (column 4) were week 11 of study for 15-week, 6-month, and 15-month old rats and week 9 for 25.5-month old rats. The numbers in parentheses equal the number of rats per group.

Abbreviations: NA = data not available; ALC = ad libitum controls; DEF = deficient; PF = pair-fed controls.

Significant differences between ALC and DEF rats of the same age were indicated by: *$P \leq 0.05$; **$P \leq 0.01$; and ***$P \leq 0.001$.

Tissue PLP

The PLP concentrations in muscle, liver, and brain are shown in *Table 2*. For each tissue, ALC and PF rats were not significantly different ($P \leq 0.05$) between age or diet groups, so PF rats were used throughout as the control group in statistical comparisons. Although plasma PLP was lower in the oldest PF rats, tissue PLP concentrations were not different. Liver and muscle demonstrated a slight decrease with age which was not significant. When tissue PLP concentrations for ALC and PF rats were combined for each age, liver PLP levels were slightly greater than muscle PLP for all ages except 25.5-month rats, while brain PLP concentrations were approximately 35% the level in muscle. Regardless of age, plasma PLP concentrations were affected most severely by vitamin B-6 deficiency, followed by, in decreasing order, muscle, liver, and brain.

The muscle PLP content of DEF rats was 36% of the 15-week PF rats, 58% of the 6- and 15-month PF rats, and 81% of the 25.5-month PF rats. These decreases in muscle PLP in B-6 deficiency were significant ($P \leq 0.01$) for all age groups.

Table 2 Pyridoxal 5'-phosphate concentration in plasma (nmol/L), liver, muscle and brain (nmol/mg protein) of rats

Group	Plasma	Muscle	Liver	Brain
15 Weeks				
ALC	1024 ± 70[a]	150 ± 2[a]	160 ± 5[a]	58 ± 2[a]
DEC	40 ± 4***[d]	52 ± 2***[b]	69 ± 4**[c]	27 ± 1**[b]
PF	775 ± 56*[b]	146 ± 5[a]	144 ± 8[a]	54 ± 4[a]
6 Months				
ALC	766 ± 40[b]	157 ± 6[a]	192 ± 5[b]	54 ± 3[a]
DEF	34 ± 3***[d]	90 ± 5**[c]	101 ± 4**[d]	33 ± 1**[c]
PF	783 ± 56[b]	156 ± 5[a]	158 ± 6[a,b]	52 ± 2[a]
15 Months				
ALC	780 ± 30[b]	149 ± 4[a]	172 ± 11[a,b]	54 ± 2[a]
DEF	110 ± 8***[e]	87 ± 6**[c]	84 ± 3**[d]	40 ± 2**[d]
PF	737 ± 38[b]	149 ± 7[a]	161 ± 11[a]	60 ± 3[a]
25.5 Months				
ALC	492 ± 89[c]	156 ± 5[a]	144 ± 13[a]	60 ± 3[a]
DEF	44 ± 5***[d]	109 ± 5*[d]	107 ± 6[ad]	43 ± 3[ad]
PF	354 ± 19[c]	135 ± 6[a]	144 ± 12[a]	56 ± 2[a]

Note. All data are given as mean ± SEM. Final plasma values (column 2) were week 11 of study for 15-week, 6-month, and 15-month old rats and at week 9 for 25.5-month old rats. Within an age group, there were no significant differences in tissue PLP between ALC and PF rats.

Abbreviations: ALC = ad libitum controls; DEF = deficient; PF = pair-fed controls.

Significant differences between DEF and PF rats of the same age were indicated by: $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

[a,b,c,d,e] For each tissue, significant differences ($P \leq 0.05$) were indicated by different superscripts.

The amount of liver PLP found in the 15-week DEF rats was 48% of the PF rats of the same age, while liver PLP of 6- and 15-month DEF rats was 64% and 52% of PF rats, respectively. These differences in liver PLP were significant ($P \leq 0.01$) for 15-week, 6-month, and 15-month rats. Although liver PLP of 25.5-month DEF rats was only 76% of 25.5 month PF rats, the difference was not significant.

The concentrations of PLP found in brain of 15-week, 6-month, and 15-month DEF rats were 49%, 63%, and 67%, respectively, of that found in the brains of PF rats of the same age group. These differences in brain PLP were significant ($P \leq 0.01$). Twenty-five and one-half-month DEF rats were only 77% of 25.5-month PF, but this difference was not significant. In the 15-week DEF rats, muscle, liver, and brain PLP were depleted greater than the other ages studied ($P \leq 0.05$).

Correlations between plasma PLP and tissue PLP concentration were examined for muscle, liver, and brain (*Figure 1,A-C*) after combining all groups. For each tissue, the equation of the least squares line, the level of significance and the coefficient of determination (R^2) were calculated for the combined population. Correlations were also significant if ages were analyzed separately (data not shown).

Glycogen Phosphorylase (GPase) Activity

Total GPase activity, the combined activity of active and inactive (activated in the presence of 5 mmol/L AMP) forms of the enzyme, is shown for muscle, liver, and brain in *Table 3*. There were no differences in GPase activity of each tissue between PF and ALC rats of any age. In PF rats, GPase activity (mean ± SEM) as an average of all ages, expressed as nkat/mg protein, was highest in skeletal muscle (7.64 ± 0.17), followed by brain (1.73 ± 0.04), and then liver (1.58 ± 0.06).

A vitamin B-6 deficiency resulted in significant de-

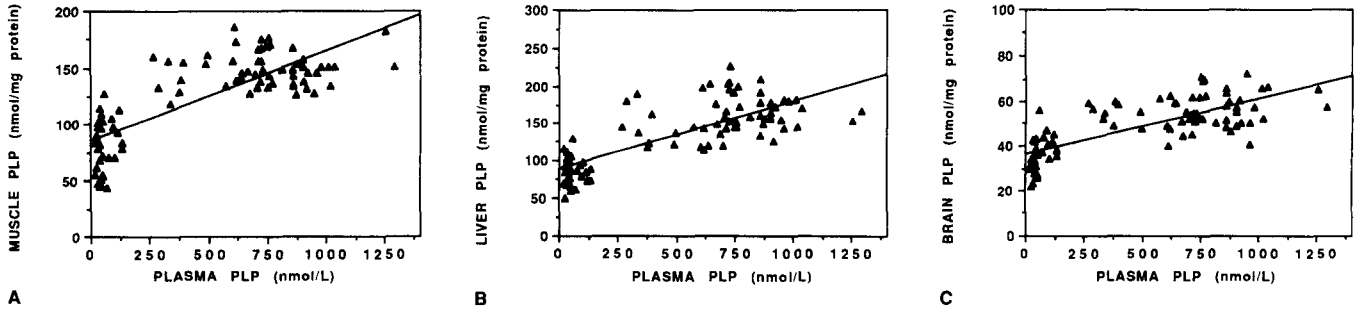

Figure 1 Correlation of tissue pyridoxal 5'-phosphate (PLP), expressed as nmol/mg protein and plasma PLP (nmol/L) using ad libitum control (ALC), pair-fed control (PF), and vitamin B-6 deficient (DEF) rats of different ages. (A) Muscle ($P \leq 0.01$, $R^2 = 0.62$); (B) Liver ($P \leq 0.01$, $R^2 = 0.60$); (C) Brain ($P \leq 0.01$, $R^2 = 0.56$).

Table 3 Glycogen phosphorylase activity (nkat/mg protein) in liver, muscle and brain of rats

Group	Muscle	Liver	Brain
15 Weeks			
ALC	8.34 ± 0.36[a]	1.44 ± 0.12[a]	1.86 ± 0.08[a]
DEF	5.34 ± 0.22***[b]	0.78 ± 0.13**[b]	1.47 ± 0.10**[b]
PF	7.78 ± 0.22[a]	1.78 ± 0.12[a]	1.78 ± 0.07[a]
6 Months			
ALC	7.82 ± 0.40[a]	1.39 ± 0.09[a]	1.73 ± 0.09[a]
DEF	6.52 ± 0.53[a,b]	1.22 ± 0.04*[c]	1.48 ± 0.06[a,b]
PF	7.85 ± 0.49[a]	1.54 ± 0.09[a]	1.59 ± 0.09[a]
15 Months			
ALC	6.92 ± 0.44[a]	1.43 ± 0.10[a]	1.70 ± 0.08[a]
DEF	6.33 ± 0.52[a,b]	1.22 ± 0.12[a,c]	1.69 ± 0.04[a,b]
PF	7.52 ± 0.32[a]	1.55 ± 0.08[a]	1.76 ± 0.07[a]
25.5 Months			
ALC	8.29 ± 0.39[a]	1.37 ± 0.13[a]	1.99 ± 0.05[a]
DEF	6.80 ± 0.37[a,b]	1.21 ± 0.11[a,b,c]	1.72 ± 0.05[a,b]
PF	7.35 ± 0.20[a]	1.37 ± 0.14[a]	1.90 ± 0.06[a]

Note. All data are given as mean ± SEM. Each group (column 1) represents 5–9 rats. Within an age group, there were no significant differences between ALC and PF rats.

Abbreviations: ALC = ad libitum controls; DEF = deficient; PF = pair-fed controls.

DEF rats were significantly different than PF rats: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$.

[a,b,c,d]: For each tissue, there were significant differences ($P \leq 0.05$) in values with different superscripts.

pression of GPase activity in muscle ($P \leq 0.001$), liver ($P \leq 0.01$) and brain ($P \leq 0.01$)) of 15-week DEF rats compared to PF rats. In 6-month DEF rats, only liver GPase activity was depressed significantly ($P \leq 0.05$). There was a depression of muscle and brain GPase in 6-month DEF rats which was not statistically significant. Although there was a decrease in GPase activity in muscle, liver, and brain of 15- and 25.5-month DEF rats, it was not statistically significant.

The correlations between tissue PLP concentration and tissue GPase activity are shown separately for muscle (*Figure 2, Panel A*), liver (*Figure 2, Panel B*), and brain (*Figure 2, Panel C*). For each tissue, the equation of the least squares line, the level of significance, and the coefficient of determination (R^2) were calculated for the combined population. Correlations were also significant if ages were analyzed separately (data not shown).

Discussion

Plasma levels of PLP have been shown to decrease with age in humans.[21-23] A similar trend was observed in this study in different aged rats fed a control diet. In many human elderly populations, a decrease in plasma PLP may be explained by a lower than average intake of vitamin B-6,[10,11] since plasma levels of PLP have been shown to correlate positively with pyridoxine intake.[24] However, the lower plasma PLP levels observed in this study for 25.5-month old rats cannot be attributed to decreased food intake since they were consuming amounts of food similar to the younger rats. The changes in blood alkaline phosphatase, albumin,[25] or decreased kinase activity[7] are all possible mechanisms by which PLP might be lowered in aged animals or humans.

In this experiment, PLP concentrations of muscle and liver were expressed in nmol/mg protein and were similar to previously reported values in the literature for rats on vitamin B-6 adequate diets.[24,26] If we assume that the rat brain is approximately 77% water from 90 days of age until death,[27] then brain PLP levels in this study can be compared to earlier reports by converting ug/g wet weight to nmol/mg dry weight. Brain PLP values of young and adult rats in this study were also similar to previously reported values.[28,29]

In vitamin B-6 deficiency, tissue PLP depletion was less marked with increasing age. These data imply that during aging there may be a decreased turnover of PLP in the tissues possibly as a result of a decrease in growth rate, protein turnover, physical activity, and metabolic rate. Li et al.[30] have shown that there is regulation of PLP concentration in liver through the binding of PLP to specific proteins, so that PLP is protected from intracellular degradation by phosphatases. The mechanism of this release and metabolism may be regulated differently in aged rats than in young growing rats. The significant correlation between plasma and tissue PLP for all aged rats implies that plasma PLP is still an accurate indicator of tissue PLP concentrations in young and old rats.

These data suggest that muscle is a more accessible supply of PLP than liver or brain since it was most responsive to vitamin B-6 depletion. The differential depletion of PLP in tissues may exist because the

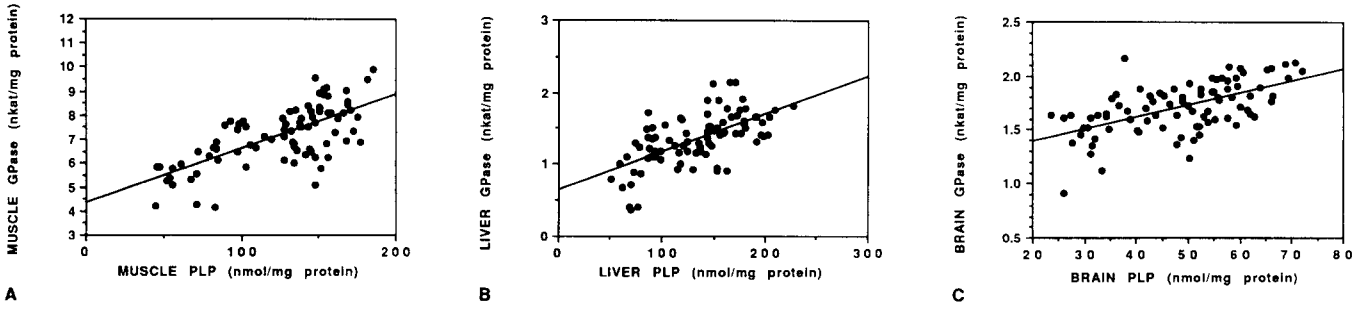

Figure 2 Correlation of tissue total glycogen phosphorylase (GPase) activity, expressed as nkat/mg protein and tissue pyridoxal 5'-phosphate (PLP) concentration, expressed as nmol/mg protein using ad libitum control (ALC), pair-fed control (PF) and vitamin B-6 deficient (DEF) rats of different ages. (A) Muscle ($P \leq 0.01$, $R^2 = 0.48$); (B) Liver ($P \leq 0.01$, $R^2 = 0.39$); (C) Brain ($P \leq 0.01$, $R^2 = 0.35$).

mechanism for storage and release of all vitamin B-6 forms is different in each tissue. It is possible that the total vitamin B-6 concentration of a tissue was not different in deficiency, but that there was an age-related shift in the distribution of the six vitamin B-6 forms. Altered vitamin B-6 metabolism during aging was demonstrated by Fonda et al.[8] who showed that less radiolabeled PLP was transaminated to PMP in the senescent mouse liver than in the young. Transamination of PMP to PLP requires the activity of an amino acid transaminase, which has been shown to decrease in the livers of aging rats.[31,32] However, Kant et al.[33] failed to demonstrate altered metabolism of vitamin B-6 in aging humans, but did report a lowered plasma PLP.

A strong association between enzyme and cofactor protects PLP-dependent enzymes from degradation and reduces the mobilization of PLP from the tissue.[30,34] When dietary vitamin B-6 decreases, the affinity of PLP for an enzyme determines PLP dissociation and mobilization. Brain PLP of the young rats decreased the same percentage as the decrease of PLP in liver, yet retained significantly more GPase activity. Liver GPase lost the greatest percentage of GPase activity with an intermediate loss of PLP, possibly because the liver contains many PLP-dependent enzymes other than GPase. These other liver enzymes may have greater affinity for PLP.[35] Muscle, which binds 90% of its PLP to GPase,[1] lost greater than 50% of its PLP during vitamin B-6 deficiency, but retained a significantly higher percentage of GPase activity than liver. Alternatively, another explanation could be a differential decrease in other PLP binding proteins in brain and liver such that there would be a differential redistribution of intracellular PLP.

GPase activity was highest in skeletal muscle, followed by brain, then liver. A similar pattern of activity was observed by previous investigators.[36,37] Although liver is known to contain large amounts of glycogen, Cori et al.[38] were the first to report GPase activity in brain. Liver would be expected to have high GPase activity, but brain, with very low glycogen stores (0.1% w/w) also demonstrated considerable GPase activity.[39] The significance of such high levels of GPase activity in brain warrants further investigation.

The effect of vitamin B6-deficiency on GPase activity was most severe in young, growing rats. Other studies, which only looked at young, growing animals, have shown a similar depression in muscle,[3] liver,[4] and brain.[5] Our data show that the degree of depression of GPase activity was dependent on the extent of PLP depletion in the tissue. Although PLP decreased 42% and 19% in 15- and 25.5-month DEF rats, respectively, the decrease was not great enough to result in a significant depression in GPase activity. GPase activity was significantly depressed in muscle and liver of 15-week and 6-month DEF rats, only. Muscle and liver of 15-week and 6-month DEF rats also demonstrated the greatest decreases in PLP.

Black et al.[40] reported that GPase decreased significantly only in extreme vitamin B-6 depletion and concluded that it was starvation, not a B-6 deficiency, which caused the decrease in GPase. In this study, DEF rats were compared to PF rats to avoid possible effects of differences in food intake. The restricted food intake resulted in significantly lower body weights in PF rats compared to ALC rats; however, there was no difference in GPase activity of ALC and PF rats. Thus, the depression observed in DEF rats was not due to decreased food consumption.

In conclusion, we wish to propose three different age-related responses to a vitamin B-6 deficiency. The youngest rats (15 weeks old), which were growing most rapidly, were affected most severely by vitamin B-6 deficiency and showed significant depletion of tissue PLP and a decrease in GPase activity. The adult rats (6 and 15 months old) demonstrated an intermediate depletion of tissue PLP, as well as an intermediate effect on GPase. Finally, vitamin B-6 deficient aged rats (25.5 months old) demonstrated a significant decrease in plasma PLP on a control diet, yet demonstrated only slight reductions in tissue PLP and GPase activity. Since each of the three age groups demonstrated a different pattern of depletion, there may be multiple mechanisms to explain the patterns of PLP depletion and decreased activity of GPase in these stages of development, growth, and aging.

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